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SOIL REACTION AND BLACK ROOT-ROT OF TOBACCO

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Black root-rot is a serious menace to successful tobacco production in the Connecticut Valley. The causal fungus is found in most tobacco soils of the Valley. Low temperature during the growing season, and high lime conditions in the soil, both stimulate its activity. Injury may vary all the way from complete destruction of the crop, on heavily limed soils in cold growing seasons, to a damage scarcely noticeable. Not only does the root-rot depress yield, but it may injure quality of leaf as well.

There are but two known methods of combating the disease: first, through control of lime conditions in the soil; and secondly, through the use of root-rot resistant strains of tobacco. This bulletin reports results of research on the former.

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SOIL REACTION AND BLACK ROOT-ROT OF TOBACCO

By P. J. ANDERSON, A. VINCENT OSMUN and W. L. DORAN[1]

Black root-rot probably causes greater loss to the tobacco growers of the United States than does any other disease. In the Connecticut Valley, according to published records, it has caused varying amounts of damage since 1906. It was probably prevalent long before that time, but had escaped attention. Within the last decade its ravages have been reduced considerably through improved cultural practices, based on a better knowledge of the effect of certain environmental factors on the virulence of the disease. Nevertheless it still takes its annual toll from the crop and, as one of the causes of "tobacco sick" soils, is an important factor in tobacco production in Massachusetts.

Symptoms of Black Root-Rot

The first symptom of the disease which the grower notices in the field is that the tobacco doesn't grow. The plants remain stunted, with narrow, thick, tough leaves and either a starved yellow color or, where the nitrogen supply is high, a very dark green color commonly called "black" by the tobacco man. On hot days the leaves wilt and "flag" more quickly than healthy tobacco plants. The dwarfed plants "top out" prematurely. Only rarely is a field equally affected in all parts. Usually there are "patches" from a square rod to several acres in extent, where the tobacco is short while in other parts of the field the growth is normal. In the diseased "patches" the plants are frequently very uneven in growth.

From the above ground symptoms, however, it is not possible for even an expert to be sure that this is black root-rot. Other troubles such as brown root-rot, lack of fertilizer, or water-logged soil may produce the same appearance. One must dig the plants and wash the soil from the roots to see the lesions which are the unmistakable signs of black root-rot. Normal young roots are white, but on a diseased plant many are black (brown at first), either throughout their length or frequently only in segments, with other segments appearing normal. Most of the ends of the small roots are black, indicating that in digging the plant, the root broke at this point and the decayed end was left in the soil. The tissue of the smaller roots is rotted through, but on the large roots there occur enlarged, rough, scurfy lesions which may or may not kill the interior tissues. Frequently the tap root is entirely rotted off at the bottom and there is an increased number of laterals. This results in a brush-work of intermingled brown, black and white small roots just above the blackened end of the main root. The brown lesions are caused by the same organism (*Thielavia basicola* Zopf)[2] which later produces the black ones, the color depending on the age of the lesion. The character which distinguishes this disease from all other tobacco diseases is the coal black color of parts of the roots. The reduced root system is unable to se-

1 During the first year of this investigation the work was conducted by G. H. Chapman, formerly research professor of Botany at this Station.

2 McCormick (Conn. Agr. Exp. Sta. Bul. 269, 1925) considers this *Thielaviopsis basicola* (Berk.) Ferraris.

cure sufficient water and soil nutrients for normal growth of the above ground parts of the plant, hence the dwarfed slow growth and "flagging" on hot days.

Black root-rot may also occur in the seed-beds, where it exhibits the same symptoms, viz., slow growth, yellow or "black" color of leaves, "flagging," and black rotted roots. Many of the plants also die. The beds look very uneven.

Previous Investigations

In the first experiment station publication which recorded and described the disease in the Connecticut Valley, Jenkins and Clinton (5:7)[3] stated:

"It is not definitely known whether an acid, alkaline or a neutral soil is best adapted to the growth of the fungus, which we know can grow in the soil itself apart from the tobacco root, but it is reasonable to suppose that this factor may have its influence."

Later (5:8):

"One of the growers whose fields suffered severely was inclined to lay it to the excessive use year after year of potash fertilizers, which gradually accumulated in the soil, and especially to the use of the carbonate of potash."

The first, and up to the present date the most important, contribution on the relation of soil reaction to root-rot was a brief circular by Briggs (1) describing results of experiments in Connecticut in 1907. His conclusions are best summarized in the following statements which we quote:

"The results of the writer's investigations thus far indicate that the tobacco is much more severely injured by the fungus on fields where the soil has been made alkaline by the long-continued use of large amounts of lime, ashes and fertilizers containing carbonate of potash."

"In all cases it was found that the plots on which carbonate of potash was used gave the smallest plants and the lowest yields. The use of lime with the carbonate of potash still further diminished the yield. The use of carbonate of potash and lime with cottonseed meal and ground fish gave a yield of but 900 pounds per acre. This yield was 200 pounds less to the acre than when no fertilizer was used and was only a little more than half the yield obtained from some of the other plots."

In pot experiments he found that:

"In soils free from root-rot, the use of 1 per cent of lime gave a better growth of tobacco than where no lime was used. This shows that the lime does not injure the tobacco directly . . . . The use of hydro-chloric acid, on the other hand, increased the yield by about 10 per cent although this acid carries no plant food . . . . The root systems of the plants in all the pots to which lime or carbonate of potash was added were badly diseased . . . . The roots of the plants grown in acid-treated soil were perfectly clean and white, and entirely free from the fungus."

All of Briggs' experiments were with naturally infested soils from fields where the soil acidity had been greatly reduced by use of alkaline fertilizers and lime. The reaction of the soil was not determined either before or after the treatments which he applied.

Johnson and Hartman (7:53-60) determined the influence of soil reaction on root-rot by growing plants in pots after adding graduated quantities of

3 Numbers in parenthesis refer to bibliography at the end of this bulletin. Numbers after the colon give the page on which statement referred to may be found.

calcium hydroxide to a naturally very acid soil inoculated with macerated diseased roots. Acidity was determined in terms of lime requirement according to the Truog method. From these experiments they concluded that:

". . . . the highest acidity practically eliminated damage from root-rot, but that heavy infection still occurred in fairly acid soil."

When they inoculated all the soils heavily with spores of the fungus *just before planting,* they found that:

"A heavy infestation apparently reduced the efficacy of the acid soils to nothing, at least in the presence of a susceptible variety. The disease appeared, in fact, more virulent in the most acid soil."

This last experiment indicates that the effect of the acid soil is not to increase the resistance of the plant but to retard the development of the fungus.

They also tested in the field the effect of application of acid fertilizers and concluded that:

"For Wisconsin conditions . . . . application of acid fertilizers to soil, alkaline or neutral in reaction, will not reduce infection by *T. basicola.*"

Chapman (2) in connection with an investigation of "tobacco sick" soils of Massachusetts made 300 determinations of the lime requirement of tobacco soils and correlated them roughly with the condition of the crop and prevalence of root-rot. He concludes (2:38) that:

"Soils with a lime requirement up to 3000 pounds CaO per acre are not producing good crops as a rule and are comparatively free from root-rots. Those with a lime requirement of from 3000 to 8000 pounds CaO per acre are in good tobacco condition; but in this group, pathogenic fungi are abundant in the soil, and the plants during certain seasons, are very liable to suffer from root-rots caused by some of these fungi. Soils with a lime requirement of 8000 pounds CaO up are usually comparatively free from such fungi, and even in unfavorable seasons, little disease is found."

He explains the relative freedom from root-rot of the soils which show the lowest lime requirement by assuming that these soils are too alkaline for the development of the fungus. His results on this point are at variance with those of Johnson and Hartman. The poor yield is attributed to the unfavorable effect of a too alkaline soil on the tobacco plant.

Chapman questioned the lime requirement method as a means of measuring the acidity of the soils.

Need of Further Investigation

We may summarize the results of all the investigations recorded up to the present: A soil made less acid by the use of lime, carbonate of potash, or ashes favors the development of black root-rot. When, however, the acidity is increased, there is a corresponding reduction in amount of root-rot injury until a point is reached where it is negligible. In regard to the particular degree of acidity which must be attained to escape root-rot and, in general, the correlation of reaction and infection throughout the soil range, Briggs gives no information while Johnson and Hartman and Chapman had contradictory results. In view of this situation and the fact that it is now commonly accepted that the development of fungi is proportionate to *intensity* of acidity as measured by the hydrogen ion concentration method, rather than the *amount* of acidity, as measured by the lime requirement method, there seemed to be need for further experimentation.

Two related problems concerned (1) the effect of lime on tobacco in the absence of root-rot and (2) the effect of the timothy cover crop.

In regard to the first, it is safe to assume that the growers considered lime beneficial, else it would not have come into such general use. Briggs found that 1 per cent of lime in disease-free soil increased growth. Hartwell and Damon (3:437) include tobacco among the lime loving plants. McCall (9) reports that, on heavy loam soil in Maryland, lime increased the yield of tobacco, but that direct applications of lime tended to lower the quality of the leaf. Hutcheson and Berger (4:7) found that, in Virginia, lime at first gave a larger yield but the tobacco was coarse and "bony." In the later years of the experiments, however, they found the tobacco reduced both in quantity and quality by lime. It is quite possible that in this case the later effects were due to root-rot rather than to the direct effect of lime. This is true in other similar experiments where the first effects were good but in later years there was a reduction. Since in most cases root-rot is not mentioned, one cannot be sure whether the experimenter was measuring the effect of the lime or of root-rot. This difficulty renders worthless most of the literature on the effect of lime on tobacco.

During the last decade, the use of timothy as a cover crop has come into considerable favor with tobacco growers of the Connecticut Valley. It reduces washing and blowing of the light soils during the long period between tobacco crops and also adds considerable humus to the soil. Some growers also have the impression that it reduces black root-rot by neutralizing in some way the ill effect of lime. When this experiment was begun there were no published records of experiments to determine the effect of its use on the succeeding crops of tobacco. It became desirable, therefore, to test by accurate experiments its immediate and cumulative effects.

The Five-Year Experiment

In 1921 a series of experiments was, therefore, started for the purpose of answering these three questions:

1. What effect does application of lime have on growth of tobacco in the absence of root-rot?

2. What is the relation between severity of infection and acidity as measured by hydrogen ion concentration?[4]

3. What is the effect of a timothy cover crop on the yield and quality of tobacco and on the severity of root-rot?

During the last five seasons Havana tobacco has been grown continuously on a field of 1.2 acres on the experiment station farm where no tobacco has been previously raised for at least 8 years. The soil is apparently of the Gloucester series. The field was divided into 24 plots of 1-20 acre each according to the diagram presented in figure I. It will be noted from this diagram that limed strips of plots alternated with unlimed strips in one direction; while at right angles, there were duplicate strips of plots (1) without

4 In measuring reaction in terms of hydrogen ion concentration method, a neutral soil or other substance is designated by the number pH 7, acid substances by the numbers below 7, alkaline substances by those above 7 up to 14. Thus a soil designated by 6 is slightly acid; 5, much more so; 4, more than 5; etc. We are not concerned here with the alkaline end of the scale since all our tobacco soils are naturally acid, and fall within the range from 4 to 7. Even when a large amount of lime is applied, they do not remain above 7 for any considerable time. The degrees of acidity are usually written pH 5, pH 4.5, etc.

cover crop (2) with timothy cover crop and (3) with clover cover crop.5. One-half of the field was inoculated while the duplicate half was not inoculated. All cultivation was in the direction of the cover crop strips, thus avoiding as far as possible carrying the fungus to the uninoculated half.

The limed plots were treated year by year as follows:

1921, ground limestone, 4000 lbs. per acre
1922, precipitated lime (51 per cent CaO), 2000 " " "
1923, agricultural lime (60 " " CaO), 4000 " " "
1924, no lime
1925, no lime

The fertilizers applied each year just before setting the plants are described in the following table.

Fertilizers Used—Pounds per Acre

Material	1921	1922	1923	1924	1925
Cottonseed Meal	1200	1200	1481	1270	828
Ammonium Sulfate	120	120	62	90	100
Dry Ground Fish	180	180	247	195	200
Acid Phosphate	160	160	.....	.....	.....
Precipitated Bone	120	120	432	276	.....
H. G. Sulfate of Potash	240	240	278	252	280
Ammo-Phos	.....	.....	.....	.....	234
Total rate per acre	2020	2020*	2500†	2083‡	1642‡

* Plus a quickly available side dressing at the second hoeing.
† Plus 250 lbs. nitrate of soda per acre applied in July.
‡ Equivalent to 3000 lbs. per acre of a 5-4-5 fertilizer.

That half of the field which was inoculated (see Figure I) was first inoculated in 1921 by setting in it diseased plants taken from a badly infested seed bed. In order to secure heavier infection on the inoculated half, a handful of soil from a heavily infested greenhouse bed was placed in the furrow where each plant was set in 1922. The inoculated half of the field was again inoculated just before setting the plants in 1923, when two cartloads of heavily infested soil from a greenhouse bed were scattered broadcast over it and harrowed in.

Timothy and clover cover crops were sown after harvesting the tobacco in 1921, 1922, and 1923. None were sown in 1924.

Soil samples for pH determination were taken from all plots each year, and the hydrogen ion concentrations which were determined are recorded in Table I.6 These soil samples were taken before the ground was broken in

5 Since the clover winter-killed every year, its effect if any must be attributed to the very small growth between sowing and freezing of the soil.

6 These and subsequent determinations were made by F. W. Morse, Henri D. Haskins, and L. S. Walker, chemists on the staff of the Massachusetts Agricultural Experiment Station. The determinations were made on air dried soils by the Clark and Lubs colorimetric method. The water extract was made by adding 200 cc. of distilled water to 20 grams of air-dry soil in a flask and allowing it to stand about 1 hour with frequent shaking. The extract was then filtered by turning the soil mixture on a small filter in a large funnel. The first portions of the filtrate were returned to the soil flask until the extract was running clear. By this time, the paper was filled with soil, and the filtration was through soil supported by a small paper. By this means, any absorption by the paper was reduced to its lowest terms.

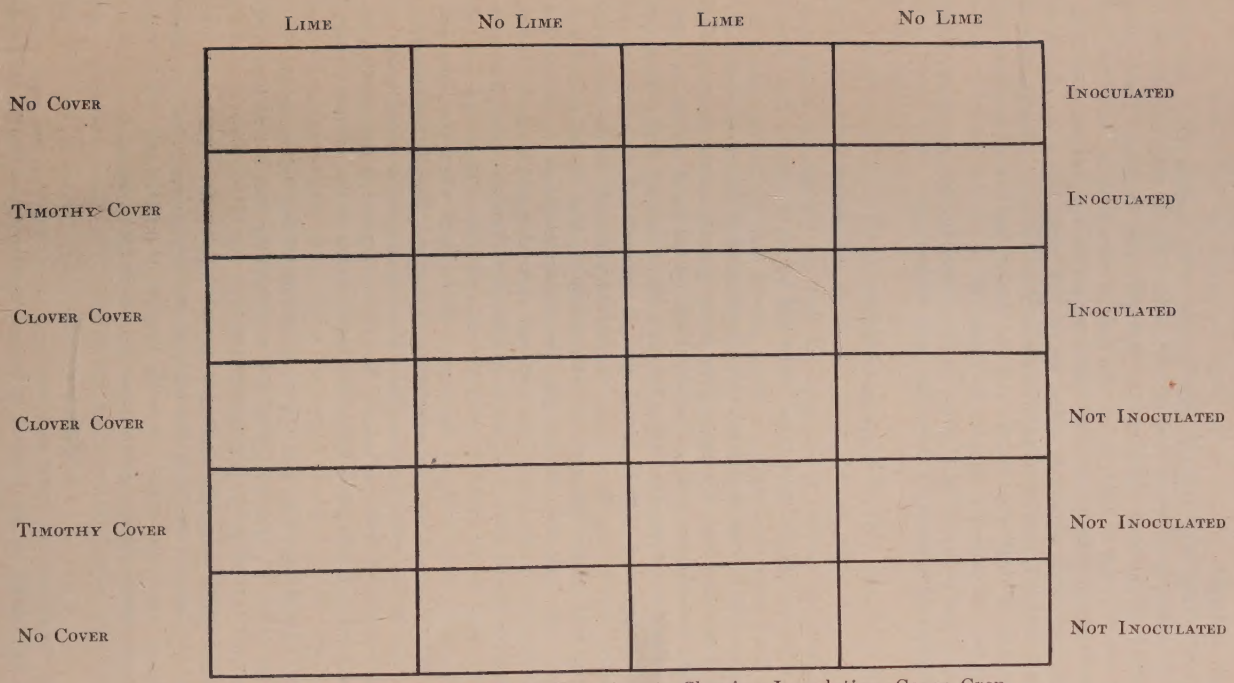

Figure 1. Diagram of Field Experiments Showing Inoculation, Cover Crop, and Lime Treatment of Plots.

May in 1922, 1923, and 1924, and in April, 1925.

During each growing season observations on growth were recorded. Immediately after the crop was harvested roots from each plot were examined for black root-rot.

No yield records were taken in 1921 because of the uneven stand, due principally to setting at different periods. When the crop of 1922 was harvested, 160 plants from the four center rows of each plot were weighed. These green weights are recorded in Table I. At the time of harvesting in 1923, 1924, and 1925, the tobacco from each entire plot, except for the borders, was hung, stripped, and cured separately. The weights of cured leaves are tabulated by plots in Table I, and the results summarized in Table II. Sorting records for the crop of 1925 were made, and the percentage for each grade and length of leaf in each plot is given in Table III.

The work and results of each of the last four years are presented separately before discussing conclusions from the whole experiment.

Season of 1922

Throughout the summer it was apparent that (1) the timothy strips were making a poorer growth than those which had no cover crop or had clover; (2) the limed plots were making a better growth than the corresponding unlimed plots both on the inoculated and uninoculated parts of the field; (3) the inoculated half was not making as much growth as the uninoculated. These field observations were all substantiated by the yield records as given in Table I and summarized in Table II. This shows that by liming there was a gain of 12 per cent both on inoculated and uninoculated plots; by the use of a timothy cover crop there was a loss of 13 per cent on the inoculated and 18 per cent on the uninoculated half; and finally that the inoculated half was 15 per cent lighter all through than the uninoculated half. After cutting the tobacco, some of the roots from each plot were dug and washed. Lesions were found on practically all of the roots from the inoculated plots, but were not much worse on the limed than on the unlimed plots. They were located especially on the large roots at the bottom of the stalk where the roots had to grow through the handful of infested earth that was deposited at planting time. A few lesions were found on the roots from the uninoculated half.

In 1922 the use of lime was associated with an increase in the yield of tobacco, and was not associated with any great increase in the amount of root-rot.

This year there was a decided loss in weight of tobacco following the timothy cover crop. The percentage of loss from timothy was not as heavy on the inoculated half as on the uninoculated half.

Inoculated plots uniformly yielded a lighter crop than uninoculated, although examination of the roots did not indicate very severe infection. Most of the injury was in the region where the handful of infested soil was in contact with the roots and not general over the whole root system.

Season of 1923

About the middle of the summer it became apparent that in the infected half of the field the tobacco on the limed strips was lagging behind the unlimed strips in growth. This difference increased throughout the summer. The

fact that a similar difference was not apparent on the uninoculated half indicated that the dwarfing effect was due to the influence of lime on the root-rot. When the roots were examined it was found that (1) the disease was very bad on all the inoculated limed plots, (2) the lesions were fairly prevalent on the roots of the unlimed inoculated plots (but much less so than on the limed plots), (3) there were frequent lesions on the uninoculated limed plots, and (4) only rarely could a lesion be found on the uninoculated, unlimed plots.

The results noted in 1923 may be summarized as follows: In the inoculated half of the field, the yield on limed plots was 16 per cent less than the yield on unlimed plots. In the uninoculated half of the field, the yield on limed plots was still 7 per cent more than the yield on unlimed plots, but this was a drop of 12 per cent from the previous year. On the inoculated half of the field even the unlimed plots suffered some loss from black root-rot, the yield being about 8 per cent less than on unlimed plots in the uninoculated part of the field.

In 1923 there was a small loss from the use of timothy, and, as in the preceding year, this loss was less on the inoculated than on the uninoculated plots.

Season of 1924

It became apparent early in the season that the limed plots were making a much poorer growth than the corresponding unlimed ones. This was particularly striking on the inoculated half, but it was also quite apparent from the poor growth that there were many places in the uninoculated half where root-rot was doing serious danage. It was plainly evident that root-rot was no longer confined to the inoculated end of the lime strips but that it was now present even to the extreme opposite end. A second very apparent fact was that there were some very good plots of tobacco (unlimed) in the inoculated part of the field, showing that the effect of very thorough inoculation was not lasting when the soil condition was not right. These observations during the growing season were substantiated by examination of the roots after the crop was harvested. It was then found that, irrespective of inoculation, the number of lesions was very small on all plots which had not been limed. On the limed strips, lesions were particularly numerous on the roots from the inoculated plots; but many of the roots from the other end were in just as serious condition, while others were relatively free. This indicated that on the uninoculated limed plots, the infestation was still uneven. Nevertheless it seemed to be spreading rapidly in these. The cured weights per acre as given in Tables I and II showed a loss from liming of 17 per cent on the inoculated plots and 12 per cent on the uninoculated plots.

In 1924, the fourth year of the experiment, the loss from black root-rot on limed plots was more severe than in the previous years, although no lime was applied this year.

In the unlimed plots, the amount of root-rot was small, whether or not the plots were inoculated. The yield on the inoculated plots was only 4 per cent less than on the corresponding uninoculated plots.

For the third successive year a reduction in yield was associated with the use of the timothy cover crop.

Season of 1925

The effect of lime on black root-rot and yield and the effect of timothy cover crop on yield were never more conspicuous than in 1925, although no cover crops had been planted and no lime applied since 1923.

Throughout the season growth was much superior in unlimed plots to growth in limed plots. It was apparent that on plots which had had a cover crop of timothy in previous years the tobacco was making a better growth than on plots which had never had a cover crop of timothy. In other words, the depressing effect of timothy which had been observed in previous years was not in evidence after this cover crop was omitted for one year. There was no visible difference in growth between the inoculated and uninoculated halves of the field. It was evident that, regardless of inoculation, black root-rot was causing loss in all limed plots.

When the roots from all plots were examined at harvest time, an average of 98 per cent of the plants in all limed plots was found to be severely infected with black root-rot. In unlimed plots an average of only 14 per cent of the plants showed symptoms of this disease, and infection on them was slight. The previous cover crop was found not to affect the amount or severity of black root-rot. Infection, as revealed by the examination of the roots, was the same on the inoculated and uninoculated parts of the field. Brown root-rot was slightly more pronounced on limed than on unlimed plots, but the difference was small.

The soil in limed plots had, in April 1925, pH values from 6.4 to 7.0. In all these, plants were severely infected and there were no visible differences between them as regards infection. The soil in unlimed plots ranged in pH values from 4.5 to 5.0. Infection in these plots was absent or slight, and there were no appreciable differences between them.

In 1925 the yield on inoculated plots was no less than the yield on plots not inoculated. The black root-rot fungus was evidently present in all plots, but had not become established in unlimed plots because of unfavorable soil conditions. It had, however, become established in limed but uninoculated plots. The black root-rot fungus is probably present in all tobacco soils, but it is evident that it causes no loss in soils having a reaction unfavorable to the fungus.

The average reduction in yield due to liming (35 per cent) was greater in 1925 than in any previous year of the experiment, although no lime was applied in either 1924 or 1925. During this two-year period without more lime added, the soil in the limed strips had, however, become slightly less acid. The omission of lime for two years on land on which tobacco was grown continuously did not result in any immediate relief from black root-rot. The greater loss in 1925 was probably not directly due to any change in the soil reaction, but to the fact that the soil is becoming more completely infested with the fungus.

The injurious action of lime as expressed in black root-rot was not more pronounced in its effect on yield than on quality and value. This is recorded in Table III under per cent darks, seconds, mediums, fillers, and brokes. The average per cent mediums and seconds on limed plots was 6, and on unlimed plots 22. The average per cent fillers on limed plots was 39 and on unlimed plots 17.

In 1924, for the third consecutive year, the use of timothy cover crop was associated with a reduction in yield. No cover crop was sown in 1924. In

1925 the plots which had previously had a timothy cover crop yielded more than plots on which a cover crop had never been grown. The average increase of plots which had had timothy cover crop over plots which had had no cover crop was 17 per cent. The increase following timothy was greater on limed than on unlimed areas. An examination of roots showed that after the omission of timothy cover crop for one year there was no significant difference between the amount or severity of brown root-rot on plots with and without timothy cover crop. The depressing effect of timothy cover crop on tobacco seems to disappear if the cover crop is omitted for but a single year.

Discussion of the Five-Year Experiment as a Whole

Occurrence of Thielavia in Tobacco Soils and Rate of Infestation

This fungus probably occurs to a limited extent in all of our soils. It has been shown that it occurs on the roots of nearly a hundred other species of plants besides tobacco (6). Many of these are common cultivated and weed plants. Further investigation would undoubtedly show that there are many other hosts. But even though its host plants were not present—they do not seem to be necessary—it apparently lives indefinitely in the soil on dead organic matter. In the examination of tobacco roots from fields which appeared quite normal and healthy above ground there are usually found occasional lesions of black root-rot. The reason that most Connecticut Valley tobacco fields do not suffer severely from black root-rot is not the absence of the fungus, but the maintenance of a soil condition which is unfavorable for its rapid propagation. Soil reaction is probably the most influential factor. An extensive survey of Massachusetts tobacco soils has not been made, but those which have been tested have been fairly acid with a reaction ranging between pH 4.5 and 5.8 except where they have been limed within a few years. Within this range there seems to be very little spread and the degree of infestation is light. But when raised above this range by application of lime or other alkaline substances, conditions are made more favorable to growth of Thielavia and the soil becomes more heavily infested. Increase in degree of infestation however is a slower process than has been commonly supposed. Most investigators of the disease have assumed that when acidity was reduced the disease would immediately become serious. In our own experiments it was not until the second year after liming was started that a high infestation was secured. We have seen a number of cases where growers have applied lime or wood ashes and the effect the first year was an increase in the yield of tobacco. It was only in the second or third year after liming that the trouble became serious. Hutcheson and Berger (4:7) noticed in their experiments that the first effect of lime was beneficial but its ultimate effect was to reduce the yield. It has been the common experience of those who have tried to produce the disease by soil inoculation that their first attempts were unsatisfactory. Since infection of the roots is local, i.e., the fungus does not travel far from the point where it enters the root, the amount of damage is in direct proportion to the number of places at which the root has been infected by the fungus. The degree of soil infestation must therefore be high before the disease becomes serious.

Similarly it is probable that a return of infested soil to a more acid condition will not be immediately followed by a great reduction in the amount of black root-rot. The results in 1925 indicate that the omission of lime and the continuous culture of tobacco do not quickly result in the disappearance of black root-rot injury. Further field work on this point is in progress.

Effect of Lime on Yield of Tobacco in the Absence of Root-Rot

It has been previously mentioned (p. 121) that certain investigators have found that the yield of tobacco was increased by liming the soil, and one of the objects of the work at the Massachusetts Agricultural Experiment Station was to determine whether such was the case under our conditions and if so, to measure the extent of increase. This was also a necessary preliminary to drawing any conclusion as to the effect of liming on root-rot. No records of yields for the first year of the experiment (1921) were made. The records of the second year show an increase of 12 per cent on both the inoculated and uninoculated parts of the field. Theoretically, this should have been larger the first year, since root-rot must have taken a slight toll the second year. During the third year the loss from root-rot more than counterbalanced the gain from lime on the inoculated part, and there was a total loss of 16 per cent. Even on the uninoculated part, root-rot was beginning to reduce the gain which would otherwise have been anticipated from liming, but there was still a final gain of 7 per cent on this half. During the fourth year of the experiment, root-rot had increased to such an extent that it more than overcame the benefit of the lime on the uninoculated part, and we had a final loss even on that side of 12 per cent as opposed to a 17 per cent loss on the inoculated part. In 1925 root-rot had increased so much that the benefit of lime was overcome even more and the loss was about 35 per cent on both the inoculated and uninoculated areas. This is a much greater loss than could have been predicted, since at the previous rate of progress the loss would have been about 17 per cent.

In answer to the first question which was proposed as the object of this experiment we may state that the immediate effect of lime in this soil was to increase the yield. In this case it was 12 per cent the second year. Undoubtedly, the degree of increase from liming depends on the reaction of the soil before application is made. On a soil less acid than this, one would hardly expect the same degree of increase.

How Acid Should the Soil Be?

It has again been demonstrated that the ultimate effect of heavy applications of lime is to increase root-rot and thus reduce yield. The facts that (1) neutral calcium salts such as land plaster do not produce this effect and (2) other alkaline substances such as carbonate of potash do produce it, show that the influencing factor is not the calcium but the alkaline character of the lime. The less acid or neutral soil thus produced does not render the tobacco plant more susceptible to attack but it favors the growth of the fungus and results in a larger number of lesions on the root system. Although there are probably no tobacco soils free from Thielavia, it is apparent that many of them, by virtue of their acid reaction, are so nearly free that their infestation is negligible; while others, by virtue of their less acid reaction, are so heavily

infested as to render profitable tobacco growing impossible. The all important question now is: Just where is the dividing line between safety and certain loss? Just what degree of acidity must a soil possess in order to be safe from root-rot?

The data on the soil and crop of 1924 are valuable in determining the location of the dividing line, because at that time the soil must have more nearly reached an equilibrium after the treatment of the three preceding years, and because this equilibrium was not disturbed by artificial inoculation or application of lime in 1924. Correlating for that year the root inspection records, the yield and sorting records and the soil reaction records, we find that root-rot was not serious on any of the twelve plots which had an average reaction of slightly over 5; but that it caused serious damage on all the inoculated and most of the uninoculated plots with an average of 5.9. By using individual plot records it may be confined more closely. Especially instructive is the record of plot 13 which kept a reaction of 5.5-5.6 throughout the three years and which was inoculated as thoroughly as any other plot in the field for three years; but on the 4th year only an occasional lesion on the roots could be found and it was the best plot in the field. Similarly, an inspection of the individual limed plots indicates pretty severe loss at 5.8.

In 1925, soil reactions were determined in April and in October. The pH values were found to have changed considerably during the growth of the tobacco in 1925. This was especially conspicuous in the limed plots, in which the soil became more acid, probably because of the removal of some of the lime by the crop. The pH values determined in April are indicative of the environment in which the crop grew. The reaction of plots without lime was between pH 4.5 and 5.0, and in these plots black root-rot caused no apparent loss. Limed plots had pH values between 6.4 and 6.9, and in all of them black root-rot caused serious damage. There were this year no plots having pH values between 5.0 and 6.4, and so the critical point cannot be located any more closely than has been done above on the basis of the results in 1924.

Experiments in pots in the greenhouse, which are to be described in a later paper, have led us to the conclusion that loss from black root-rot occurs whenever the pH value of the soil is 5.9 or higher, and that loss from black root-rot does not occur at pH 5.6 or below, except in exceptionally cold soils.

We may *therefore conclude that the critical point is somewhere between 5.6 and 5.9.* It is apparent however that there cannot be in reality a critical *point* but rather a critical *region*, below which damage from root-rot may be expected to be little if any, and above which root-rot is almost certain to cause trouble. There are at least three reasons why this region cannot be defined more accurately than the limits given above:

1. The transition from a soil reaction unfavorable for growth of Thielavia to a reaction which favors is a matter of degree—not of plus and minus.

2. During a very warm summer the critical region would probably be higher since a higher soil temperature reduces the effect of root-rot, as has been shown experimentally by Johnson and Hartman (7:60). A cold summer on the other hand would probably depress the critical region toward the acid range.

3. On compact, poorly drained soil black root-rot is commonly worse than in looser soil in the same field, due probably to the difference in the rate of leaching and to lower soil temperature. This also is in accord with the experimental results of Johnson and Hartman (7:80). It is not improbable that

on such a soil the location of the critical region would be lower. On the basis of the yields of the last three years it may be stated that, as the acidity of the soil becomes less above 5.9, the loss caused by black root-rot becomes greater. (The upper limit of alkalinity has not been determined.) Plots on the limed areas in this experiment may be placed in four groups on the basis of the pH values of their soils. These are correlated with average yields as follows:

pH	5.9 to 6.1	—	yield	1233	pounds
"	6.1 " 6.2	—	"	1183	"
"	6.2 " 6.3	—	"	1120	"
"	6.3 " 6.4	—	"	1039	"

We have, then, quantitative evidence of the increasing injury from black root-rot which results when by the addition of the lime the soil is made less and less acid to a point beyond pH 5.9.

How Much Lime May Be Used with Safety?

If the soil has reached such a point, and if it is seriously infested with the black root-rot fungus, what then may be done? If no further lime is applied and if tobacco is continuously grown on such soil, the loss of lime by leaching and by removal in the plant will gradually result in a more acid condition. Of course the loss from black root-rot may be so great as to make tobacco growing on such land unprofitable. Continuous tobacco, no further liming, and the use of acid fertilizers, such as sulfate of ammonia and sulfate of potash, are all the remedial measures to be suggested at this time. A quicker method of increasing soil acidity is desirable, and this matter is now under investigation.

The Effect of Timothy

Since timothy is extensively used and widely recommended as a cover crop for tobacco soils, it was assumed at the outset that it was known to increase the yield of tobacco by virtue of the organic matter which was added to the soil or the plant food which was saved from leaching through its use. It was included in the present experiment to see whether it influenced the damage from black root-rot and as a check against the clover cover crop which was supposed to increase root-rot because it is a host plant of Thielavia. At the end of the first season, therefore, it was a surprise to find that both the inoculated and uninoculated timothy plots showed a reduction in yield—13 per cent and 18 per cent on the inoculated and uninoculated plots respectively. It could not have been a result of the timothy depleting the soil water because 1922 was an extremely wet year. Losses from the use of timothy both on the inoculated and uninoculated plots, but in a smaller degree, were also recorded in 1923 and 1924. Certain plots at times showed small increases over adjacent plots without cover, but considering the experiment as a whole, the conclusion is inescapable that the effect of timothy has been to decrease both the yield and value of the tobacco. This is in agreement with data recently published by Jones (8) who reports an average decrease in yield of 13 per cent associated with the use of the timothy cover crop. The percentages

of loss during two of the years were not so high on the inoculated as on the uninoculated plots, but the reverse was true the third year. Certainly there was not, on the whole, sufficient evidence from either the yield data or the examination of the roots to prove that timothy had any influence on the prevalence or severity of black root-rot.

In 1925, after one year's omission of cover crops, the plots which had had timothy cover crops yielded 17 per cent more than the plots which had never had cover crop. It would appear from this that the depressing effect of timothy cover crop quickly disappears and is replaced by a beneficial effect when the cover crop is not sown for one year. This increase following timothy after an interval of one year was greater on limed than on unlimed plots. In this connection it is interesting to consider the previous depressing effect of timothy on limed versus unlimed plots. For the three years previous to 1925, the average reduction in yield associated with the use of timothy cover crop was 5 per cent on limed plots and 10 per cent on unlimed plots.

When the roots of the plants on the timothy plots were compared with those on the adjacent plots without cover in years previous to 1925, it was found that many of the laterals—especially the lower ones—were brown and dead. The symptoms were quite typical of the disease which is now commonly called brown root-rot. The disease was not entirely lacking in any of the plots but was certainly more pronounced on the timothy plots. On the timothy plots which had been limed, black root-rot was also present, and it was not always possible to separate the influence of the two; but on the unlimed plots the brown root-rot was unmistakable. Brown root-rot, then, is apparently one of the causes of depressed yields on the timothy plots. It is known that timothy in rotation encourages this disease, and it is not surprising that we should now find the same effect from timothy used as a cover crop. This, however, is not a satisfying explanation, because we do not yet know what brown root-rot is. Whether or not it is caused by soil organisms or by toxins or malnutrition has never been determined. We are not even sure that all the tobacco-sick soil troubles which we include under the title of brown root-rot are the same. The only characters they have in common are the presence of some dead brown roots and consequent reduced growth.

Pure Culture Experiments on the Relation of H-ion Concentration to Growth of Thielavia

From the preceding, it is apparent that the adverse effect of lime on tobacco is due to the favorable influence of an alkaline reaction on the growth of Thielavia. In order to see whether this conclusion is supported by growth of the fungus in pure culture, synthetic culture media (Czapek's) were prepared and adjusted to the following reactions: 1.5, 2.0, 3.05, 3.45, 3.8, 3.9, 4.25, 4.55, 5.35, 6.05, 6.2, 7.2, 7.9, 8.55, 8.9, 9.6, 9.9, 11.05, 12.1, and 12.9. The reactions were determined both colorimetrically and electrometrically. Three series of cultures were made, one in tubes and the others in Petri dishes. Records of measurements and notes on growth were made at intervals of 3 or 4 days. Briefly summarized, the results were: On all cultures between 6.05 and 12.9 the growth consisted of a dense black mat of mycelium on the surface and dark hyphae under the agar. Both chlamydospores and conidia were produced in great abundance. Within this range no difference in character or quantity of growth could be observed. Apparently the reaction was

optimum throughout this entire range. But below this it was otherwise. At 5.35 the rapidity of growth began to decrease. Below 5.35, all growth on the surface ceased and even under the surface it was progressively more scanty down to 3.05. Below this, growth ceased altogether. The production of chlamydospores also decreased progressively with the scantiness of growth until there were none at all at 3.05. A few conidia were produced even to the most acid point where mycelium grew.

Since in pure culture the fungus will grow even at reactions which are more acid than any tobacco soils, it is pertinent to ask why we do not have loss from root-rot below 5.5. The answer is probably to be found in the difference between conditions which the fungus encounters in a sterile artificial medium and what it encounters in its natural medium, the soil. In the latter case it meets keen competition from other soil organisms and it is only the strong vigorous growers that get the available food and thrive. But, as was noted above, Thielavia at 5.35 is beginning to lose its vigor and at more acid ranges it dwindles to nothing. When in its weakened condition due to unfavorable reaction it is being crowded out by other organisms better adapted to acid conditions, it is not strange that Thielavia should be able to make but little headway at 5.35 or lower. Incidentally it should be kept in mind that Thielavia does occur sparingly on roots in more acid soils, and the lesions are just as pronounced but are not numerous enough to cause much loss. The critical point at which vigor of growth begins to diminish is thus lower than 6.05 and higher than 5.35. Thus it must fall somewhere in the region which we have called the critical region, and the results from the pure culture tests support those of the field tests.

Summary

1. The immediate effect of liming an acid soil was to increase the yield of tobacco.

2. If sufficient lime is added, black root-rot is ultimately promoted, and there is consequently a reduction in yield.

3. Black root-rot is present in most soils but is more injurious in nearly neutral soils because the causal fungus, *Thielavia basicola*, grows more rapidly and vigorously in a nearly neutral medium.

4. In the field under experimentation, black root-rot caused little or no loss in soils more acid than pH 5.6. It caused severe loss on all soils less acid than pH 5.9. It should be understood that this critical region between pH 5.6 and 5.9 is an intermediate zone which may be shifted to a somewhat higher or lower position by variations in temperature and compactness of soil, and by differences in the degree of infestation of the fungus in the soil. Growth of the fungus in pure culture on media confirmed the results in the field.

5. As the soil becomes less acid above pH 5.9, the loss from black root-rot increases. The upper limit of alkalinity has not been determined.

6. After repeated applications of lime brought the soil reaction to a point favorable to the fungus, the omission of lime for two years, with tobacco grown continuously meanwhile, did not result in any immediate reduction in the severity of black root-rot.

7. If, because of extreme acidity, lime is considered necessary, it should be applied with great caution and in small applications, always preceded by determinations of soil reaction in order to avoid approaching too closely the danger point of pH 5.9.

8. The use of timothy cover-crop was found to have no significant effect on the prevalence of black root-rot.

9. The use of timothy cover-crop reduced the yield of tobacco for three consecutive years. This effect was associated with brown root-rot.

10. When timothy cover crop was omitted for one year, the depressing effect of timothy quickly disappeared and was replaced by a beneficial effect. In these experiments the yield on plots so treated exceeded by 17 per cent those without cover crop.

Literature Cited

1. Briggs, L. J. The field treatment of tobacco root-rot. U. S. Dept. Agr. Bur. Plant Indus. Circ. 7: 5-8. 1908.
2. Chapman, G. H. Tobacco investigations, progress report. Mass. Agr. Expt. Sta. Bul. 195: 1-38. 1920.
3. Hartwell, B. L. and S. C. Damon. The comparative effect on different kinds of plants of liming an acid soil. R. I. Agr. Expt. Sta. Bul. 160: 407-446. 1914.
4. Hutcheson, T. B. and D. J. Berger. Experiments with bright tobacco and other crops grown on bright tobacco farms. Va. Agr. Expt. Sta. Bul. 233: 3-19. 1923.
5. Jenkins, E. H. and G. P. Clinton. Root-rot of tobacco. Conn. Agr. Expt. Sta. Bul. of Immediate Information 4: 1-11. 1906.
6. Johnson, James. Host plants of Thielavia basicola. Jour. Agr. Res. 7: 289-300. 1916.
7. Johnson, James and R. E. Hartman. Influence of soil environment on the root-rot of tobacco. Jour. Agr. Res. 17: 41-86. 1919.
8. Jones, J. P. Havana seed tobacco as influenced by timothy cover crop. Mass. Agr. Expt. Sta. Circ. 73: 1-3. 1925.
9. McCall, A. G. Fertilizers for Maryland soils. Md. Agr. Expt. Sta. Bul. 247: 117-151. 1922.

TABLE I. Treatment, Yields, and Reaction Analyses by Plots throughout the Five Years of the Root-Rot Experiment

Plot	Inoculation	Cover Crop 1921–24	Lime in May 1921	pH May 1922		Lime in May 1922	Green Weight 160 Plants Sept. 1922 Lbs.	pH May 1923		Lime in May 1923	Cured Leaves Lbs. per Acre 1923	pH May 1924		Cured Leaves Lbs per Acre 1924	pH April 1925		Cured Leaves Lbs. per Acre 1925
1		None		5.0			396	5.2			1231	4.9		1453	4.6		1131
2	+	Timothy	None	5.2	Average 5.11	None	201	6.0	Average 5.56	None	1372	5.2	Average 5.01	1320	4.6	Average 4.76	1362
3		Clover		5.1			345	5.2			1358	4.9		1449	5.0		1339
4		Clover		5.1			349	5.5			1590	4.8		1468	5.0		1184
5	—	Timothy		5.2			348	5.7			1457	5.0		1528	4.8		1237
6		None		5.0			387	5.8			1670	5.3		1608	4.6		1187
7		None		5.4			323	6.2			976	6.0		1150	6.9		696
8	+	Timothy	Ground Limestone 2 tons per acre	6.2	Average 5.73	Precipitated Lime 1 ton per acre	353	6.3	Average 6.06	Agricultural Lime 2 tons per acre	891	5.8	Average 5.9	1315	6.7	Average 6.7	1050
9		Clover		6.2			349	6.2			1216	6.0		1182	7.0		748
10		Clover		5.6			388	5.7			1599	6.0		1324	6.4		766
11	—	Timothy		5.5			374	6.2			1373	5.7		1500	6.5		1005
12		None		5.4			477	5.8			1500	5.8		1354	6.7		632
13		None		5.6			378	5.5			1472	5.5		1621	4.7		1492
14	+	Timothy	None	5.8	Average 5.70	None	348	5.6	Average 5.68	None	1245	5.0	Average 5.03	1354	4.8	Average 4.72	1344
15		Clover		5.7			301	5.6			1486	4.9		1566	4.8		1196
16		Clover		5.8			430	5.6			1641	5.0		1563	4.7		1303
17	—	Timothy		5.7			356	6.3			1269	5.2		1522	4.8		1433
18		None		5.4			441	5.5			1231	4.6		1488	4.5		1273
19		None		5.3			393	6.1			1132	5.8		1278	6.5		820
20	+	Timothy	Ground Limestone 2 tons per acre	6.0	Average 5.77	Precipitated Lime 1 ton per acre	391	6.0	Average 6.05	Agricultural Lime 2 tons per acre	1245	6.6	Average 5.9	1117	6.7	Average 6.65	888
21		Clover		6.5			394	6.2			1415	5.8		1257	6.7		796
22		Clover		5.5			471	6.0			1886	5.8		1313	6.4		732
23	—	Timothy		6.0			388	6.0			1316	5.8		1207	6.9		994
24		None		5.3			486	6.0			1401	5.6		1437	6.7		714

TABLE II. Summary of Yields for 1922, 1923, 1924, and 1925

Pounds per Acre

Treatments	1922 Green	1923 Cured	1924 Cured	1925 Cured
Average of all limed plots:				
Inoculated	7340	1146	1217	868
Not inoculated	8620	1579	1356	807
Average of all unlimed plots:				
Inoculated	6560	1361	1461	1319
Not inoculated	7700	1476	1535	1269
Average of timothy cover plots:				
Inoculated	6460	1193	1277	1161*
Not inoculated	7320	1354	1439	1167*
Average of plots without cover crop:				
Inoculated	7450	1203	1375	1034
Not inoculated	8960	1450	1472	951
Average of all plots:				
Inoculated	6960	1253	1338	1076
Not inoculated	8160	1494	1443	1038
Percentage Gain (+) or Loss (—)				
From liming:				
Inoculated	+12	—16	—17	—34
Not inoculated	+12	+ 7	—12	—36
From timothy cover:				
Inoculated	—13	— 1	— 7	+12*
Not inoculated	—18	— 7	— 2	+23*
From inoculation:				
Limed	—15	—27	—10	+ 8
Unlimed	—15	— 8	— 5	+ 4

*Timothy not sown in 1924, no cover crop plowed under in 1925.

TABLE III. Sorting Record of Crop of 1925

Plot	Treatment	pH*	Yields per Acre Lbs.	Darks		Seconds		Mediums		Fillers	Brokes
				Per cent	Length Inches	Per cent	Length Inches	Per cent	Length Inches	Per cent	Per cent
	No Lime:										
1	No cover crop	4.6	1131	50.9	15-25	24.9	15-25	0.6	Not sized	15.8	7.8
2	Timothy	4.6	1362	56.0	15-25	18.7	15-23	None		16.0	9.3
3	Clover	5.0	1389	49.3	15-27	22.9	15-23	None		21.5	6.3
4	Clover	5.0	1184	53.6	15-25	17.9	15-23	0.9	21-23	20.4	7.0
5	Timothy	4.8	1237	51.6	15-25	21.7	15-23	2.5	21-25	17.8	6.4
6	No cover crop	4.6	1187	51.2	15-27	21.1	15-25	2.8	21-25	17.8	7.1
	Lime:										
7	No cover crop	6.9	696	59.8	15-21	0.5	15	None		39.7	None
8	Timothy	6.7	1050	44.4	15-21	27.9	15-21	None		27.0	0.7
9	Clover	7.0	748	46.5	15-21	3.0	15.19	None		48.6	1.9
10	Clover	6.4	766	54.9	15-21	1.9	15-19	None		43.2	None
11	Timothy	6.5	1005	65.6	15-23	5.3	15-19	None		27.5	1.6
12	No cover crop	6.7	632	41.7	15-23	1.5	15-19	None		56.1	0.7
	No Lime:										
13	No cover crop	4.7	1492	56.8	15-27	23.5	15-25	0.6	Not sized	13.6	5.5
14	Timothy	4.8	1344	55.6	15-27	16.6	15-23	None		17.2	10.6
15	Clover	4.8	1196	50.8	15-27	22.3	15-23	0.6	Not sized	16.4	9.9
16**	Clover	4.7	1303	54.9	15-27	14.7	15-25	2.2	21-25	15.4	12.5
17	Timothy	4.8	1433	51.5	15-27	21.5	15-23	1.6	21-25	17.5	7.9
18	No cover crop	4.5	1273	51.2	15-27	19.2	15-25	2.6	21-25	17.5	9.5
	Lime:										
19	No cover crop	6.5	820	51.9	15-21	5.6	15-19	None		41.6	0.8
20	Timothy	6.7	888	63.1	15-21	9.3	15-19	None		27.1	0.5
21	Clover	6.7	796	46.5	15-21	6.0	15-19	None		45.0	2.5
22	Clover	6.4	732	44.6	15-23	3.9	15-19	None		50.5	0.9
23	Timothy	6.9	994	52.9	15-23	13.7	15-21	None		26.6	6.6
24	No cover crop	6.7	714	38.7	15-23	2.9	15-19	None		56.6	1.5

*April, 1925. **0.3 per cent (3.9 lbs.) overdamp.